

Effect of *Glomus mosseae* on various host to record colonization, spore production, soil pH and soil temperature

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ABSTRACT

Mycorrhizal fungi were species that intimately associate with plant roots forming a symbiotic relationship with the plants providing sugar for fungi and fungi providing nutrients such as phosphorus to the plants. Mycorrhizal fungi accumulate phosphate and transport large quantity of phosphate within their hyphae release to plant cell in root tissue. The present investigation entitled as effect of *Glomus mosseae* on various host to record colonization, spore production, soil pH and soil temperature was conducted at Plant Pathology Section, College of Agriculture, Nagpur, for mass multiplication of VAM ten different host was taken for study such as follows guinea grass (*Panicum maximum*), para grass (*Urochloa mutica*), napier grass (*Pennisetum purpureum*), marvel (*Dichanthium annulatum*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.), bajara (*Pennisetum typhoideum*), pea (*Pisum sativum* L.), uninoculated control. Out of the ten host guinea grass (*Panicum maximum*) responded as most suitable host showing highest colonization 87.66 per cent and 420 spore production. It was observed that plants having higher AM colonization showed AM production showing a positive correlation. As time advances the intensity VAM colonization and spore production was increased upto 90 days. Soil pH and soil temperature did not change during investigation.

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INTRODUCTION

Vesicular arbuscular mycorrhizal fungi are ubiquitous plant root symbionts that can be considered as keystone mutualists in terrestrial ecosystem forming a link between biotic and abiotic components via carbon and nutrient fluxes, that pass between plant and fungi in the soil (Oneill *et al.*, 1995).

The presence of arbuscular mycorrhiza are symbiotic fungi found associated with roots of a wide range variety of plant species. In natural communities approximately 80 per cent of higher plants are obligatorily dependent on fungal associates and 18 per cent typically non-mycorrhizal. Arbuscular mycorrhiza fungi role in symbiotic partnership is provided with a

fine hyphal network capable of extending the range of root hairs (Allen, 1991). The fungus receives many benefits including increased nutrient absorption. This means that in the plant world mycorrhizal symbiosis is the rule rather than exception. (Harrison, 2005) The fine threads that make up the fungus branch between soil particles grow into decomposing organic matter even explore the shells of dead insects where they find phosphorus and other vital nutrients are passed back to the root of plants.

Vesicular arbuscular mycorrhiza allows the fungal symbionts to extend a greater amount of nutrients from the soil such as phosphorus nitrogen, zinc, boron and colonized by arbuscular mycorrhizal fungi benefits plant in a number of ways increased disease resistance, enhanced water relation and increased soil aggregation (Gerdemann, 1975; Hayman, 1982 and Newsham *et al.*, 1994).

Therefore, the studies were undertaken *in vitro* and *in vivo* for the evaluation of suitable hosts for mass multiplication.

MATERIAL AND METHODS

The present investigation entitled effect of *Glomus mosseae* on various host to record colonization, spore production, soil pH and soil temperature was carried out *in vitro* and *in vivo* in the laboratory of Plant Pathology Section, College of Agriculture, Nagpur during 2013 - 14.

The following materials, equipments were used for the research work in the plant pathology laboratory college of agriculture Nagpur.

Collection of host crop :

Different types of host crop such as pea, jowar, bajra, maize and wheat were collected from local market and guinea grass, para grass and napier grass were collected from Nagpur Agriculture College Farm and inoculums of *Glomus mosseae* were collected from Plant Pathology Section, College of agriculture, Nagpur.

Glasswares :

The glassware's used during the course of research work were of Borosil make. The glassware's *viz.*, test tube, slides, cover slips, beaker and conical flask of 250 ml, 500 ml, 1000 ml funnel, Petri dish and pipettes.

Equipments :

Standard laboratory equipments *viz.*, autoclave, hot air oven, digital weighing balance, stereoscopic binocular microscope, sieves of 45 μm , 75 μm , 250 μm , 710 μm , mortar and pestle, razors and measuring cylinder etc. were used.

Preparation of reagent :

Chemicals like potassium hydroxide (KOH) and hydrochloric acid (HCl) were used for root clearing and staining. Ten g of potassium hydroxide pellets was dissolved in 100 ml water for preparation of 10 per cent KOH solution. For preparation of 0.1 N HCl solutions, 8.3 ml HCl was dissolved in 992 ml of water.

Selection of host plant :

To select the most suitable host plant species *viz.*, sorghum, bajara, maize, wheat, napier, paragrass, marvel, guinea grass, pea were tried for mass production of *Glomus mosseae* similarly traditional substrate (sand : soil) was selected for mass multiplication of *Glomus mosseae*. Pea used as uninoculated control. All host plants were selected on the basis of their suitability to the agro climatic condition of the area having thick root system for sizeable sporulation and colonization.

Pot and potting mixture :

Pots of size 35×25 cm were selected for the experiment. Pots were filled with sand soil (1:3) in the control pots only sand soil mixture raised. The substrates were thoroughly mixed before adding inoculums. A layer of inoculum consisting of AM colonized root pieces and soil containing spores 30/100g was spread over the pot mixture.

Surface disinfection and sowing of seeds :

Healthy seeds of all host plants were surface sterilized with 10 per cent solution of sodium hypochlorite for 1-2 minutes and washed several times with sterilized distilled water to remove sodium hypochlorite before sowing. Seeds were planted directly in the pots. Each treatment with different host was replicated thrice.

Multiplication and maintains of AM fungi :

The pots are regularly watered for maintains and multiplication of AM fungi.

Estimation of VAM root colonization :

Root segments each approximately 1 cm long was selected at random from a stained sample. Root colonization was studied by rapid clearing and staining technique (Phillips and Hayman, 1979 and Kormanik *et al.*, 1979) are as follows :

- Roots were washed with tap water remove soil particles then roots were cut into 1cm length by sterilized razors.
- Root pieces were placed in a small beaker with enough 10 per cent KOH solution.
- The beaker with root pieces in 10 per cent KOH solution was autoclaved at 1.04kg/cm².
- The KOH solution was decanted from the beaker leaving roots behind.
- Roots were rinsed with about 20 ml distilled water.
- Twenty ml of 0.1N HCl was added in the beaker shake left for a minute.
- HCl was decanted.
- Sufficient amount of cotton blue or trypan blue solution was added to cover the root generously.
- The roots bits were observed under microscope then the vesicular arbuscular mycorrhizal infection was counted and the per cent colonization was calculated by following formula:

$$\text{Per cent colonization} = \frac{\text{Total number of colonized root pieces}}{\text{Total number of root pieces examined}} \times 100$$

Isolation of AM fungal spore :

Spores of arbuscular fungi were isolated by using the wet sieving and decanting method described by the Gerdemann and Nicolson (1963). The procedure used was as follows.

- Hundred g soil samples (composite of 3 plants of each host crop) was taken and dissolved in 1000 ml distilled water in conical flask.
- Then conical flask was shaken for 30 minutes.
- After that the conical flask was kept in undisturbed condition for 15 minutes.
- The heavier particle was allowed to settle down.
- Suspension was decanted through 710 µm sieve to remove organic matter and roots.
- Then the suspension was decanted through 250 µm, 75 µm and 45 µm sieves consequently.
- The entire residues were collected on 45 µm sieves.

- After settlement residue was dissolved in distilled water and filtered through filter paper.
- This paper was spread in Petri dish and residue present on filter paper was taken and mounted on slide and was examined under microscope. The spore was counted by following formula :

$$\text{Number of spore in 1 g soil} = \frac{\text{Number of spore counted}}{\text{Weight of soil}} \times 100$$

Soil pH :

- The root particles were removed from soil.
- Twenty g air dry soil was grinded by using mortar and pestle.
- Then the soil was added in beaker.
- Fifty ml distilled water was added in beaker and was stirred for 1 hr.
- The pH of soil sample was measured by the pH meter.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Effect of *Glomus mosseae* on colonization, spore production, soil pH and soil temperature after 30 DAS, 60 DAS and 90 DAS :

The data given in Table 1 revealed that all the treatments were found to be statistically significant over control after 30 DAS. Treatment 9 was found significantly superior all over the treatment. The highest root colonization (63.33%), spore production 160 spores, soil temperature 22.34°C and soil pH 7.3 was recorded in the T₉, followed by T₂, root colonization was 49 per cent and spore production 131, soil temperature was 22.15°C and soil pH 7.3, followed by T₁ root colonization was 43.67 per cent, spore production 126 spores, soil temperature 22.00°C and soil pH 7.4, followed by T₇ root colonization was 42 per cent, spore production 110 spores, soil temperature 22.31°C and soil pH 7.3. Lowest colonization and spore production, was recorded in uninoculated control *i.e.* 16 per cent and 32 spores. Soil pH and soil temperature was not influenced remain constant in all host plants. The data given in table evident that all the treatments were found to be statistically significant over control after 60 DAP. Treatment 9 significantly superior all over the treatment followed

Table 1 : Effect of *Glomus mosseae* on colonization, spore production, soil pH and soil temperature after 30 DAS, 60 DAS and 90 DAS

Treatment No.	Botanical names	Common names	VAM colonization (%)	Spore production: 100 g of soil	Soil pH	Soil temperature (°C)	VAM colonization (%)	Spore production per 100 g of soil	Soil pH	Soil temperature (°C)	VAM colonization (%)	Spore production per 100 g of soil	Soil pH	Soil temperature (°C)
T ₁	<i>Urochloa mutica</i>	Para grass	43.67 (41.63)	126	7.4	22.00	57.00 (49.02)	298	7.50	22.00	73.00 (58.79)	348	7.53	22.14
T ₂	<i>Pennisetum purpureum</i>	Napier	49.00 (44.42)	131	7.3	22.15	64.00 (53.13)	305	7.40	22.15	76.33 (60.89)	395	7.46	22.16
T ₃	<i>Dichanthium annulatum</i>	Marvel	37.67 (37.85)	77	7.4	22.27	41.00 (39.81)	244	7.50	22.27	63.66 (52.93)	283	7.53	22.10
T ₄	<i>Pisum sativum</i>	Pea	24.67 (29.75)	56	7.3	22.14	28.33 (32.15)	221	7.53	22.14	46.66 (43.08)	244	7.43	22.17
T ₅	<i>Sorghum bicolor</i> (L.) Monech	Sorghum	31.00 (33.80)	88	7.4	22.18	47.67 (43.66)	261	7.53	22.18	65.33 (53.93)	290	7.53	22.31
T ₆	<i>Pennisetum typhoides</i>	Bajra	36.67 (37.23)	94	7.3	22.13	54.00 (47.29)	267	7.40	22.13	69.66 (56.58)	306	7.53	22.45
T ₇	<i>Zea mays</i> L.	Maize	42.00 (40.39)	110	7.3	22.31	63.67 (52.93)	288	7.46	22.31	71.00 (57.41)	335	7.53	22.28
T ₈	<i>Triticum aestivum</i>	Wheat	27.33 (31.50)	72	7.3	22.41	39.00 (38.64)	235	7.40	22.41	55.00 (47.86)	256	7.56	22.34
T ₉	<i>Panicum maximum</i>	Guinea grass	63.33 (52.73)	160	7.3	22.34	72.33 (58.27)	320	7.46	22.34	87.66 (69.98)	420	7.56	22.40
T ₁₀	<i>Pisum sativum</i> L. Control	Control	16.00 (23.55)	32	7.4	22.15	24.20 (29.77)	43	7.46	22.15	26.00 (30.64)	54	7.56	22.18
	F test	-	Sig.	Sig.	NS	NS	Sig.	Sig.	NS	NS	Sig.	Sig.	NS	NS
	S.E.±	-	0.79	2.26	7.34	22.20	0.39	1.71	7.34	22.20	0.51	0.43	7.46	22.25
	C.D (P=0.05)	-	2.34	6.68	-	-	1.16	5.05	-	-	1.52	1.28	-	-

(Figure are in parantheses are arcs sine transformed values). NS=Non significant

by the treatment 2, treatment 1 and treatment 7. Percentage of vesicular arbuscular mycorrhizal colonization highest in treatment 9, 72.33 per cent, spore production was 320 spores, soil pH 7.4 and soil temperature 22.34°C, followed by treatment 2 vesicular arbuscular mycorrhizal colonization 64 per cent, spore production 305 spores, soil temperature 22.15°C and soil pH 7.40. Percentage of root colonization variable due effect environmental factor, spore production was varied due soil moisture, soil microbial activity. Lowest colonization and spore production, was recorded in uninoculated control *i.e.* 24.20 per cent and 43 spores. Root colonization and spore production was positively correlated with each other. Treatment 1 percentage of root colonization were 57 per cent, spore production 298, soil pH 7.5, and soil temperature 22 °C. In treatment 7 the percentage VAM colonization 63 per cent, spore production 288 spores, soil pH 7.5, soil temperature 22.31°C was recorded. Significant difference was observed in spore production and colonization over uninoculated control. Soil pH and soil temperature was not change it remain constant after 60 days.

The data given in Table 1 revealed that all the treatments were found to be statistically significant over control after 90 DAS. T₉ superior all over the treatment followed by T₂, T₁, and T₇. The highest root colonization was recorded in T₉ *i.e.* 87.66, spore production 420 spores/100 g of soil, Soil pH 7.56, and soil temperature was 22.40, followed by T₂, where colonization was 76.33 per cent, spore production 395 spore/100 g of soil, soil pH 7.46 and soil temperature 22.14°C. In case of treatment T₁ Colonization 73 per cent, spore production 348/100 g of soil, soil pH 7.53 and soil temperature 22.14° C was recorded. Where as in treatment T₇ VAM colonization was 71 per cent, spore production, 325/100 g of soil, Soil pH 7.53 and soil temperature 22.28°C was found. Lowest colonization and spore production was recorded in uninoculated control plant. No effect of soil pH and temperature was observed on colonization and spore production.

Similar findings were also recorded by the Bauer *et al.* (2003) reported that Mycorrhizal colonization were levels in root of individuals species ranged from 3 to 90 per cent were large in member of poaceae family. Sharma *et al.* (2005); Tahat *et al.* (2008); Tanwar *et al.* (2010); Javid (2008); Parmar *et al.* (2013); Mala *et al.* (2010); Kaushish *et al.* (2011) and Channasava and Lakshman

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